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Interference with Axonal Transport of Neurofilament as the Common Etiology and Pathogenesis of Neurofibrillary Tangles, Amyotrophic Lateral Sclerosis, Parkinsonism-Dementia, and Many Other Degenerations of the CNS: A Series of Hypotheses

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(Perspectives for Research)

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Epidemiological study of the high incidence foci in the Western Pacific of amyotrophic lateral sclerosis (ALS), parkinsonism-dementia (PD), and early appearance of neurofibrillary tangles (NFTs) [1,2] has indicated a common etiology and pathogenesis for all three phenomena. The covariation of these three apparently unrelated but associated neuropathological phenomena in the high incidence foci on Guam and the Kii Peninsula of Japan and in West New Guinea (where we do not yet know about neurofibrillary tangles in the population) has indicated that an environmental factor underlies all three phenomena [3-8]. We would like to have a single mechanism of pathogenesis for neuronal degeneration in the three diseases which neurologists do not ordinarily consider to be related: ALS (motor neuron disease), PD, and Alzheimer's disease (or senile dementia of the Alzheimer type).

We are forced to this conclusion by the close covariation of the three diseases in place and in time. In Saipan, Tinian and the Northern Mariana Islands, the Chamorro people do not experience them as they do on Guam and Rota [9]. The Auyu and Jakai suffer from ALS and PD only when they live along certain river drainages [3-6]; the Japanese only in a few isolated valleys [10]. As ALS recedes so does PD on Guam [7], the Kii Peninsula of Japan [10], and in West New Guinea [6].

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Environmental deficiencies of calcium and magnesium in people who have no outside source of these elements have been the only unusual environmental factors discovered which are the same in all three high incidence foci [11-18]. The disappearance of these diseases after a lag of over a decade in all three foci, coincident with increased access to calcium from the outside, further confirms the role of calcium deficiency and the induced secondary hyperparathyroidism in pathogenesis [6,12,19-22]. It has been verified by environmental analyses of soil and water [11], presence of osteoporosis in the affected populations [23], and the discovery of calcium and other metals in soft tissues [24-31], particularly neurons [32-35] of the patients. The Raman spectra of the deposits [16] and their stability and immobility [32,33,35] confirm the specificity of these deposits in the form of stable hydroxyapatites [16], and indicate they are not formed postmortem and their presence only in neurons which bear NFTs strongly suggests their pathogenic role in the neuronal damage [32-35].

The cause of ALS and PD and the early appearance of NFTs in these high incidence foci is, thus, essentially solved: interesting and important problems of individual variation of response remain, as they do also for explaining which individual gets goiter, deaf mutism, mental retardation, or full cretinism in an iodine deficient area, or who gets general paresis or tabes dorsalis from untreated infection with Treponema pallidum.

In these three foci of high incidence ALS and PD and associated NFTS the etiology has been discovered and the mechanism of pathogenesis demonstrated from the following evidence:

1. epidemiology of occurrence and disappearance of the diseases in time and place [4,7,10];

2. environmental analyses of soil and water led to and substantiated Yase´s hypothesis [12];

3. calcium and other metal analyses of tissues, especially brain, confirm the hypothesis [25-33, 35];

4. access to calcium has resulted, after an appropriate delay, in slow disappearance of the phenomena in certain villages in all three foci [4,7,10], as access to iodine eliminates the problem in an endemic goiterous and cretinism area.

The brilliant use by Asao Hirano of his good fortune in obtaining an early autopsy on a case of early ALS has suddenly given us the basic pathological mechnism we have been seeking: he demonstrated swollen motor neurons in the spinal cord in a patient with pure ALS in New York, and found the neuronal perikaryon filled with a piled-up mass of neurofilament [36,37] which proved to be the 10 nm neurofilament by immunospecificity [38].

We have been searching for a basic pathogenesis that would account for the association of all three phenomena: NFT, ALS and PD. We may now suggest a basic pathogenetic pathway common to many different diseases and insults to the CNS: namely, interference with axonal transport of 10 nm neurofilament down the axon, without interruption of its synthesis and assembly in the perikaryon. Such interference leads to deformation of the neurofilament into paired helical filaments and neurofibrillary tangle formation and, when it occurs at different rates, to neuritic plaque formation or to amyloid formation and its deposition into plaques.

Such a mechanism may operate in pugilistic encephalopathy, in congenital genetic defects of Down´s syndrome, in hydroxyapatite deposition as in neurons in Guamanian ALS and PD, in metal intoxication as in dialysis dementia, in intoxication of rats with AlCl3 or β,β'-iminodipropionitrile (IDPN) or in early motor neuron disease (Hirano´s case) [36,37]. Precisely which specific neurons are affected depends on host factors we cannot yet discern (no more than we can in tertiary syphilis or in CNS lesions from congenital iodine deficiency), but in neurons with large demands on axonal transport (such as motor neurons) sudden ballooning and death with piled-up neurofilaments may ensue, while in neurons with less voluminous axonal transport (such as the hippocampal neurons) NFTs develop slowly.

The strange appearance of a specific autoantibody to 10 nm axonal neurofilament in scrapie, kuru, and Creutzfeldt-Jakob disease (CJD) [39-43], and in a significant but lesser portion of ALS, PD, Alzheimer´s disease, Parkinson´s disease, and other degenerations of the CNS [40,41,43,44], has led to the focus of our attention on the three proteins, having molecular weights of 200,000, 150,000, and 70,000 daltons [41,45] which comprise the 10 nm neurofilament, especially since autoantibodies against the thousands of other brain proteins are not formed in these diseases. The live neuron cultures used as substrate to detect these autoantibodies [43,46,47], readily detect other antibodies specifical-

ly directed against other brain proteins, as indicated by the use of these cultures for detection of monoclonal antibodies from hybridomas [48]. Why, then, is it specifically the neurofilament which excites autoantibody production and not other brain proteins?

The NFTs of Alzheimer's disease and senility, like those found in pugilistic encephalopathy, Down's syndrome, or certain slow virus infections, are composed of paired helical filaments, which are distinguishable ultrastructurally from normal 10 nm neurofilaments [49]. However, autoantibody, which appears in CJD and in other neurodegenerative diseases, against normal 10 nm neurofilament also reacts with paired helical filaments in NFTs of Alzheimer's disease [45], with the piled-up neurofilaments of IDPN-poisoned rats [41,50], and with the piled-up neurofilaments in motor neurons of the spinal cord in Hirano's patient with early ALS [36,38]. Furthermore, antibody prepared to normal 10 nm neurofilament reacts with the NFTs of Alzheimer's disease [45] as well as with the piled-up accumulations of 10 nm neurofilaments in brain cells of rats poisoned with $AlCl_3$ [51] or IDPN [41,50]. Thus, normal 10 nm neurofilament and paired helical filaments share idiotopes and probably arise from the same source, and CNS amyloid may well be a further accumulation of degraded neurofilament [52-61].

In the high incidence foci of the western Pacific, deposits of hydroxyapatites in neurons as a result of CNS maturation in the presence of the altered metabolism of calcium deficiency-induced secondary hyperparathyroidism appears to have been the mechanism for this slow disturbance of axonal transport of the neurofilament. Interference with transport of 10 nm neurofilament down the axon may lead to its piling up and lysis of motor neurons, or to paired helical filament formation in NFTs in more slowly metabolizing neurons. At times this piled up protein complex, especially the 200,000 dalton component of the protein triad of which neurofilaments are built, elicits an autoimmune response in prespecified B cells [39-44].

The nonantigenic, replicating unconventional slow viruses causing scrapie, kuru, and CJD evoke amyloid deposition in the brain of many, but not all, patients and some of the species or breeds of experimentally infected animals [52,62-68]. These viruses are unusually resistant to physical and chemical inactivation [62-65, 69-72], and appear to lack a nucleic acid [62-65,72,73] or a non-

host protein antigen [62-65,69,74]. Merz et al [75-79] have identified by electron microscopy of density gradient sedimented brain preparations a scrapie associated fibril (SAF) in all patients and affected animals, but not in other diseases [75,76,79]. These SAFs are 2-stranded amyloid-like fibers about 100 nm long, which increase in quantity with virus titer [75,77]. The two strands are each composed of two spiral protofilaments some 2-6 nm in diameter, as are normal neurofilaments, paired helical filaments, and amyloid fibrils. They may be the infectious agents [75,77]. They stain with Congo red and show birefringence, as do amyloid fibers which they resemble ultrastructurally [80]. They are also very similar to paired helical filaments of NFTs, yet they can be distinguished morphologically from both paired helical filaments and amyloid fibers by the degree of coiling and the regularity of their periodic narrowings [75,79]. The scrapie associated protein of Prusiner [68,73,80-82] of under 30,000 daltons molecular weight may aggregate to form these infectious SAFs [80,81,83], but such in vitro aggregates have not yet been shown to be infectious.

Thus, we now have in these unconventional viruses a possible link between normal 10 nm neurofilament and amyloid, and it appears that the SAFs are filamentous viruses which bring to mind filamentous phage fd and filamentous plant viruses which are of about the same diameters [63-65,72]. These neurofilament-like viruses apparently destroy the slow axonal transport of neurofilament down the axon and occasionally lead to amyloid accumulations. It is tempting to assume that in neurons with high transport requirements (i.e., motor neurons) an accumulation of neurofilament in the perikaryon, proximal to the axonal process, causes cell death as in Hirano´s cases of ALS [36,37], whereas in neurons requiring less axonal transport, paired helical filaments develop to form NFTs or neuritic plaques, or even amyloid plaques [50,52-59,63-65,84,85]. These viruses seem to be parasites of this slow axonal transport mechanism.

These hypotheses are but a framework of thought. They do however reflect a synthesis of much of our recent data and clearly indicate numerous new lines of inquiry which may validate or refute these predictions.

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